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Immunotherapy of brain cancer

Patrick Roth[1]*, Matthias Preusser[2], Michael Weller[1]

[1]Department of Neurology and Brain Tumor Center, University Hospital Zurich and University of Zurich, Switzerland; [2]Department of Medicine I, Comprehensive Cancer Center CNS Unit (CCC-CNS), Medical University of Vienna, Austria

*Correspondence: Dr. Patrick Roth, Department of Neurology, University Hospital Zurich, Frauenklinikstrasse 26, 8091 Zurich, Switzerland, Tel.: +41 (0)44 255 5511, Fax: +41 (0)44 255 4380, E-mail: patrick.roth@usz.ch

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Abstract

The brain has long been considered an immune-privileged site precluding potent immune responses. Still, because of the failure of conventional anti-cancer treatments to achieve sustained control of intracranial neoplasms, immunotherapy has been considered as a promising strategy for decades. Various efforts aimed at exploiting the immune system as a therapeutic weapon but were largely unsuccessful. The situation only changed with the introduction of the checkpoint inhibitors which target immune cell receptors that interfere with the activation of immune effector cells. Following the observation of striking effects of drugs targeting CTLA-4 or PD-1 against melanoma and other tumor entities, it was recognized that these drugs may also be active against metastatic tumor lesions in the brain. Their therapeutic activity against primary brain tumors is currently being investigated within clinical trials. In parallel, other immunotherapeutics such as peptide vaccines are at an advanced stage of clinical development. Further immunotherapeutic strategies that are currently under investigation comprise adoptive immune cell transfer as well as inhibitors of metabolic pathways involved in the local immunosuppression frequently found in brain tumors. Thus, the ongoing implementation of immunotherapeutic concepts into clinical routine may represent a powerful addition to the therapeutic arsenal against various brain tumors.

Immunology in the central nervous system

The central nervous system (CNS) has long been considered an immunoprivileged site because of the limited presence of immune cells under physiological, that is, healthy conditions. Trafficking of immune cells into and from the CNS is limited and controlled by the blood brain barrier (BBB) [1]. The BBB also hampers penetration of antibodies as well as plasma proteins into the CNS. Antigen-presenting cells (APC) such as microglial cells are probably less effective in priming T cells than APC outside the brain [2]. Furthermore, the expression of *Major Histocompatibility Complex* (MHC) molecules on cells in the CNS is limited which may further impede the induction of immune responses. The BBB, however, is disrupted under various pathological conditions such as inflammation and, at least partially, in various brain tumors such as metastases, high-grade gliomas or primary CNS lymphomas resulting in efflux of fluid and proteins into the brain parenchyma [3]. In this situation, migration of immune cells in the brain is possible and strong immune responses, even resulting in damage to CNS tissue, occur in multiple sclerosis and other inflammatory conditions [4]. More recent findings from rodent models point to the presence of lymphatic vessels in the CNS [5]. Furthermore, trafficking of immune cells between the meninges and the cerebrospinal fluid (CSF) has been reported which further stresses the assumption that immune cell priming in cervical lymph nodes and subsequent migration to the brain may be possible [6]. Tumor-infiltrating lymphocytes are frequently found in brain tumors and are associated with survival times in patients with brain metastases [7]. Thus, while still a part of the body with a particular immunological situation, the CNS is not isolated from the immune system. As a consequence, mounting immune responses against brain tumors might be possible and represents a promising therapeutic approach. Immunotherapeutic strategies against neoplasms in the CNS which have been explored within the last years or are currently being investigated are summarized in this review.

Cytokines

Cytokines play an important role in the activation of the immune system. Their therapeutic administration has long been considered a promising approach, particularly in the context of melanoma, a rather immunogenic tumor. Accordingly, various cytokine-based treatments have been employed within the last decades, however, without a particular focus on brain metastases. Treatment with cytokines such as interleukin (IL)-2 typically resulted in insufficient anti-tumor activity and significant toxicity. One large retrospective series investigated the effect of IL-2 in patients with melanoma brain metastases. Only minor clinical benefit was observed [8]. Another small series of 8 patients reported progressive disease in all but one patient pointing to an insufficient anti-tumor activity of single IL-2 treatment [9]. Because of the emergence of more advanced immunotherapeutics, cytokines have not been pursued extensively as therapeutic agents within the last years.

Targeting the immunosuppressive microenvironment

The microenvironment of many malignant brain tumors is dominated by immunosuppressive signals [10]. This has been investigated in detail in glioblastoma, a tumor which is paradigmatic for tumor-associated immunosuppression, because of the presence of multiple soluble and membrane-bound factors as well as immune cells with immunosuppressive properties in the tumor microenvironment [11-17]. The immune cell receptors cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and programmed cell death (PD)-1 are expressed on T effector cells and contribute to the impaired cellular immune activity against primary and secondary brain tumors (see below for details). Targeting one or more immunosuppressive signals may represent a therapeutic option either alone or in combination with vaccination. Most efforts in glioblastoma have focused on the inhibition of transforming growth factor (TGF)-β, the master immunosuppressive cytokine secreted by glioma cells. While active in various preclinical models, all approaches tested in human patients so far, have not shown therapeutic benefit or were associated with poor tolerability [18,19]. Immunosuppressive pathways which are active in gliomas and other types of cancer

comprise tryptophan depletion which is conferred by tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO). The metabolization of tryptophan as well as increased levels of the major metabolite, kynurenine, inhibit the function of immune effector cells [20] and recruit regulatory T cells with immunosuppressive properties to the tumor [21]. Targeting IDO and/or TDO may therefore represent a promising therapeutic strategy which is currently under clinical investigation [22]. Within a phase I trial, the IDO inhibitor indoximod was administered together with temozolomide in patients with recurrent glioblastoma [23]. The compound was considered safe but its anti-tumor activity needs to be investigated in larger trials.

Immune checkpoint inhibitors

Therapeutic targeting of CTLA-4, PD-1 or the ligand PD-L1 has emerged as a novel and powerful weapon against various tumors [24,25]. The available drugs have revolutionized the field of cancer immunotherapy and may also confer clinical benefit in patients with CNS tumors. However, it must be emphasized that large trials assessing the activity of these drugs against primary and secondary brain tumors are largely lacking. Thus, despite promising data from preclinical studies [26,27] as well as small or uncontrolled clinical series, the overall value of checkpoint inhibitors for patients with brain tumors still needs to be established. The current state of development of drugs targeting CTLA-4 or the PD-1 pathway in various brain tumors is summarized below.

Targeting CTLA-4

Ipilimumab is a fully humanized monoclonal antibody targeting CTLA-4. It is the first checkpoint inhibitor that was approved for patients with advanced melanoma [28]. Similar to many other trials, patients with brain metastases were not allowed to enter the initial trials or were underrepresented which did not allow for a conclusion on the activity of the drug in the brain. Following the success of ipilimumab against melanoma manifestations outside the brain, a phase II, multicentre, open-label study was initiated for patients with brain

metastases from melanoma. A total of 72 patients were enrolled with 51 patients being neurologically asymptomatic (cohort A). In contrast, 21 patients had neurological symptoms which required treatment with steroids (cohort B). Ipilimumab was administered at a 10 mg/kg dose every 3 weeks for 4 doses. Patients who did not experience tumor progression received maintenance treatment at 12 week intervals. Whole-brain radiation therapy had been administered to 33% (17/51 patients) in cohort A and 24% (5/21 patients) in cohort B before they received treatment with ipilimumab. Treatment was overall well tolerated and the most frequent side effects were diarrhea, nausea, headache, fatigue, pruritus and rash. No specific central nervous system toxicities were observed. In cohort A, 9 patients (18%) achieved partial response or stable disease while only one patient (5%) in cohort B had complete response and no other responses were noted. Assessment of responses exclusively in the brain revealed that 12 patients (24%) in cohort A and 2 patients (10%) in cohort B achieved disease control defined as complete response, partial response, or stable disease after 12 weeks. Median overall survival of patients from cohort A was 7 months and 3.7 months in patients treated within cohort B. Overall survival at 2 years was 26% for patients who were neurologically asymptomatic at study entry compared to 10% of the patients who had symptomatic brain metastases [29]. While this is the first larger study suggesting that checkpoint inhibition may also confer benefit to patients with brain tumors, various questions remain open such as the combination of immunotherapeutic approaches with standard treatment options like radiation therapy or chemotherapy. The latter was addressed in a study assessing the activity of ipilimumab in combination with fotemustine, a nitrosourea, frequently used for the treatment of tumors in the brain because of its ability to cross the BBB. The patient population of this open-lable, single-arm phase 2 trial consisted of 86 patients with advanced, unresectable stage III or stage IV melanoma of whom 20 had asymptomatic brain metastases at study entry. The rationale for this trial was that chemotherapy-induced release of tumor antigens might amplify the anti-tumor activity of ipilimumab. The treatment regimen consisted of ipilimumab given every 3 weeks to a total of four doses in combination with fotemustine. Patients with a clinical response were eligible for

maintenance treatment with ipilimumab and fotemustine. Disease control, defined as complete response, partial response, or stable disease, was achieved by 40 patients, 10 of them with brain metastases. Toxicity was common with 47 patients (55%) experiencing grade 3 or 4 adverse events. Myelotoxicity was the most frequent side effect followed by hepatic toxicity [30]. Because of the lack of a control arm, it remains to be determined whether the observed responses rather reflect fotemustine or ipilimumab activity. A phase 3 trial addressing this question is currently ongoing (NIBIT-M2, NCT02460068).

A retrospective analysis of 38 patients with melanoma brain metastases treated with ipilimumab demonstrated a partial response in 3 patients and stable disease in 5. Median overall survival was 101 days. These rather disappointing results were attributed to the late time point at which treatment with ipilimumab was initiated [31]. A retrospective series assessed the activity of radiotherapy alone or in combination with ipilimumab in 70 patients with melanoma brain metastases. Despite various confounding factors, the results point to an improved outcome when the 2 treatment modalities were combined [32]. Data of a similar retrospective series support this perception [33]. In a single-institution study, the safety and efficacy of ipilimumab in combination with stereotactic radiosurgery was evaluated in patients with melanoma brain metastases. Ipilimumab was administered before, during or after radiosurgery. Grade 3 or 4 toxicity was noticed in 20% of patients. The authors concluded that the combination is safe and may be associated with improved outcome [34]. Several studies which explore the combination of radiotherapy and ipilimumab as a treatment for patients with melanoma brain metastases are ongoing or have completed accrual but the results are pending (NCT01703507, NCT02097732).

Targeting PD-1/PD-L1

PD-1 is expressed by tumor-infiltrating lymphocytes in melanoma brain metastases [35]. Within the same publication, expression of PD-L1 by melanoma cells metastatic to the brain was reported. Although speculative, it must be assumed that the expression of PD-L1 in melanoma manifestations in the brain is predictive for response to PD-1- or PD-L1-targeted

treatment. Based on the clinical success of drugs directed against the PD-1/PD-L1 axis, the expression of the receptor and its ligand were assessed in brain metastases of various other tumor entities [36]. Although frequently present, expression levels vary between different histologies which may result in different responses to PD-1 or PD-L1 antagonists. Unprecedented results have been achieved in melanoma patients who were treated with the anti-PD-1 antibodies nivolumab or pembrolizumab alone or in combination with ipilimumab [37,38]. Still, trials focusing specifically on patients with brain metastases are lacking so far and the therapeutic activity of anti-PD-1 antibodies against brain metastases has only been explored superficially so far. First reports suggest that this treatment is not associated with unique toxicity in the CNS and may be associated with clinical benefit [39]. A phase 2 study (NCT02085070) explored the safety and activity of pembrolizumab in patients with previously untreated or progressing melanoma brain metastases. An interim analysis revealed that among 12 evaluable patients, responses of brain metastases were noticed in 3, stable disease was achieved in 2 patients. Side effects were rare with one patient experiencing grade 3 liver toxicity. An analysis of the full trial population is outstanding [40]. Non-small cell lung cancer (NSCLC) is frequently associated with the occurrence of brain metastases. A phase 2, single arm trial assessed the activity of nivolumab in patients with advanced squamous NSCLC. Four patients had brain metastases at study entry and responses to nivolumab treatment were observed. However, not further details were provided [41]. Pembrolizumab was explored in a phase II study in patients with advanced NSCLC with at least one brain metastasis previously untreated or progressing after prior local therapy. Patient who required immediate local treatment or administration of steroids were not eligible. Presence of PD-L1 expression in the tumor tissue was required for study entry. Preliminary results demonstrate that the response rate for brain metastases was 44% in 9 patients who could be evaluated. Systemic response rate was 34%. No grade 3 or 4 toxicities were reported [42]. Enrolling into this trial (NCT02085070) is ongoing and the results of a larger cohort of patients need to be awaited. A trial exploring the activity of nivolumab in patients with brain metastases from NSCLC is also ongoing (NCT01454102).

A retrospective analysis of data from two prospective nivolumab protocols enrolling 160 patients with metastatic melanoma focused on patients with brain metastases treated with stereotactic radiation within 6 months of receiving nivolumab. Toxicity was mild and no grade 3 or 4 toxicity was observed in 26 patients. Local control of brain metastases following radiation therapy at 6 and 12 months was 91% and 85%, respectively. These preliminary data suggest a good tolerability of the combination of radiation therapy and nivolumab administration [43].

Current efforts aim at further boost anti-tumor immune responses using combinations of different checkpoint inhibitors. NCT02374242 is a randomised phase 2 trial run by the “Anti-PD1 Brain Collaboration (ABC)” which assesses the activity of the anti-PD1 antibody nivolumab alone or in combination with ipilimumab in patients suffering from melanoma brain metastasis. A cohort 1 will enroll patients with asymptomatic and previously untreated brain metastases. This will be followed by a cohort 2 which is open for patients with previously treated brain metastases that have progressed after local treatment, and/or patients suffering from neurological deficits caused by brain metastases. Another trial will explore the combination of ipilimumab and nivolumab in patients with active melanoma brain metastases (NCT02320058).

Drugs targeting PD-1 or PD-L1 are now also being investigated in the context of primary brain tumors, mainly glioblastoma, in which these molecules are frequently expressed [44,45]. A phase III trial which compared the PD-1 antibody nivolumab with bevacizumab in patients with recurrent glioblastoma has completed accrual (NCT02017717). Overall survival at 6 months after initiation of nivolumab treatment was 70% in a small safety lead-in cohort of this trial [46]. Currently, PD-1 inhibitors are being tested in patients with newly diagnosed glioblastoma. Here, standard temozolomide-based radiochemotherapy will be compared with the combination of radiotherapy and nivolumab in a phase III trial for patients with glioblastoma harboring an unmethylated MGMT promoter (Checkmate 498, NCT02617589). A companion trial will be available for patients with MGMT promoter-methylated glioblastoma (Checkmate 548, NCT02667587). Other drugs which target the PD-1 pathway such as

pembrolizumab (NCT02337491) or the PD-L1 inhibitor MEDI4736 (NCT02336165) are also in clinical testing in patients with newly diagnosed as well as recurrent glioblastoma (Table 1).

Vaccination

Mounting immune responses against an established tumor by administration of a vaccine is a therapeutic strategy which has been mainly pursued in the context of glioblastoma, the most frequent malignant primary brain tumor. Early studies were primarily assessing vaccines on the basis of tumor cell lysate that was used to pulse dendritic cells (DC). DC are professional antigen-presenting cells which are supposed to initiate and boost immune cell responses. These vaccination strategies were almost exclusively tested in single-arm trials which precluded any final conclusion on their anti-tumor activity. Furthermore, with increasing knowledge on the immune system's function, it has become increasingly clear that vaccines which are produced on the basis of tumor cell lysate may not always confer a therapeutic benefit. One major obstacle of lysate-based vaccines is the presence of tumor antigens derived from self-proteins which may be subject to peripheral and central immunological tolerance mechanisms and thereby preclude powerful immune responses [47]. Accordingly, despite more than 2 decades of research on tumor lysate-based vaccines, only limited progress has been made [48]. As a consequence, this strategy has been largely abandoned in favor of more specific vaccination approaches which mainly use peptide-based vaccines. Furthermore, the search for mutated tumor antigens, exclusively present in tumor cells such as isocitrate dehydrogenase (IDH)-1 mutations (see below for details), may help to define more appropriate targets for successful vaccination strategies.

Single peptide vaccines

The clinically most advanced peptide vaccine is rindopepimut which is composed of a peptide derived from the mutant vIII variant of the epidermal growth factor receptor (EGFR) that is conjugated to keyhole limpet hemocyanin (KLH), a large immunogenic carrier protein. Rindopepimut is administered intradermally together with granulocyte-macrophage colony-

stimulating factor (GM-CSF) as adjuvant. EGFRvIII is expressed in approximately 25% of all glioblastomas. Thus, only patients with EGFRvIII-positive tumors are eligible for rindopepimut treatment. Rindopepimut has been mainly assessed in single arm trials in patients with newly diagnosed glioblastoma. Despite the conceptual limitations of such trials, the results have been regarded as very promising [49-51]. Throughout all trials, the vaccine was very well tolerated except for mild injection side reactions. Compared to historical controls, median overall survival was markedly prolonged in all studies pointing to an anti-glioma activity of this approach. Loss of EGFRvIII expression in most patients with accessible recurrent tumor tissue following vaccination suggests that immune responses against EGFRvIII were mounted successfully. However, these findings also indicate that immunoediting occurred in response to vaccination which allowed further growth of the tumor as a consequence of immune escape. A multicenter, phase III randomized trial exploring the activity of rindopepimut in patients with newly diagnosed EGFRvIII-positive glioblastoma has completed accrual (ACT IV; NCT01480479). In March 2016, a press release of Celldex indicated that the study was not further pursued because no difference between the rindopepimut arm (median OS 20.4 months) and the placebo control (median OS 21.1 months) became apparent (HR 0.99; www.celldex.com). In the setting of recurrent glioblastoma, rindopepimut was investigated in a randomized phase II study. All patients received treatment with bevacizumab and rindopepimut or a placebo vaccine was added. Median overall survival was 11.6 months in the rindopepimut arm compared to 9.3 months in the bevacizumab alone group (p=0.0386; HR 0.57 for the intention-to-treat (ITT) population) [52]. Whether anti-angiogenic agents such as bevacizumab may facilitate or rather attenuate anti-tumor immune responses is still a matter of debate. Reduced permeability of the BBB following treatment with bevacizumab may preclude trafficking of immune cells and penetration of antibodies. In contrast, vascular endothelial growth factor (VEGF) has immunosuppressive properties and blocking its function may help mounting immune responses [53,54]. Thus, the combination of anti-angiogenic drugs and immunotherapeutics needs further investigations. Other single vaccines which rely on single peptides have only been tested in smaller trials.

A peptide of the Wilms tumor peptide 1 (WT-1) was explored in patients with newly diagnosed glioblastoma and considered safe [55]. An approach which is currently assessed in clinical trials is vaccination against a mutant version of IDH-1 which is frequently found in lower grade gliomas. Promising results have been observed in a preclinical model [56]. However, the activity of this approach in human patients needs to be confirmed in ongoing trials (NCT02193347, NCT02454634).

Multi-peptide vaccines

The concept of peptide vaccination which uses a single antigen has been challenged by the fact that immunoediting may occur which allows the tumor to grow following loss of target antigen expression. Accordingly, vaccines have been developed which rely on several antigens which may at least theoretically increase the probability of mounting an immune response against one or several targets and preclude immediate immune escape due to loss of antigen expression. A multi-peptide vaccine consisting of peptides derived from several glioma-associated antigens has been assessed within 2 phase 1 studies in children and adults, respectively [57,58]. The approach was considered safe and antigen-specific T cell responses were observed. The clinically most advanced multi-peptide vaccine is ICT-107 which consists of patient-derived DC pulsed with 6 glioma-associated peptides deduced from glycoprotein 100 (gp100), melanoma-associated antigen 1 (MAGE1), absent in melanoma 2 protein **(**AIM-2), human epidermal growth factor receptor 2 (HER2/neu), IL-13Rα2, and tyrosinase related protein-2 (TRP-2). A phase 1 trial indicated that the administration of ICT-107 is safe [59]. A subsequent phase 2 trial was negative for the primary endpoint. However, a detailed analysis revealed that HLA-A2-positive patients may benefit from the vaccine, particularly those with tumors harboring a methylated MGMT promoter [60]. Accordingly, a phase 3 trial has been set up which is only open for patients who are HLA-A2 positive (STING, EORTC 1507; NCT02546102). IMA-950 is a multi-peptide vaccine which contains 11 HLA-binding tumor-associated antigens (TAA). It is administered together with GM-CSF and imiquimod as well as a single dose of cyclophosphamide which is given with the

intention to deplete regulatory T cells (NCT01920191). The GAPVAC protocol tries to establish a patient-tailored vaccine based on high-throughput analyses of the individual tumor tissue (NCT02149225). It needs to be awaited whether such expensive approaches are feasible and result in clinical benefit.

Additional immunotherapeutic approaches

Various other immunotherapeutics have been assessed mainly in patients with glioblastoma because of the urgent need for novel therapies [61]. Compared to the class of immune checkpoint inhibitors as well as peptide vaccines, only few other immunotherapeutics have reached advanced stages of clinical development or are even only at the level of preclinical testing. A DC-based vaccine using pp65 RNA administered after prior tetanus/diphtheria (Td) toxoid application was used in a phase I trial and yielded promising progression-free and overall survival times [62]. This approach needs to be explored in larger studies in order to judge its activity. Adoptive immune cell therapy has been considered a promising strategy based on results obtained in animal models. Immune cells, isolated either from the blood or the tumor, are engineered ex vivo in order to strengthen their anti-tumor activity. Among the innovative techniques which are reaching clinical development in neurooncology are chimeric antigen receptors (CAR). These proteins are composed of an antigen-binding fragment of an immunoglobulin which is linked to immunostimulatory domains and expressed in immune effector cells, e.g., T cells. Immune cells which are equipped with CAR may have a higher lytic activity against tumor cells than standard immune cells [63]. However, the use of CAR depends on the availability of tumor-specific antigens which are largely absent in many brain tumors. Not surprisingly, CAR T cells targeting EGFRvIII have been in the focus of preclinical studies [64] and first clinical studies are ongoing (NCT02209376). CAR T cells targeting IL13Rα2 were assessed in a small series of patients using local delivery of the cells into the resection cavity [65]. Larger trials need to be conducted in order to determine the clinical utility of such approaches.

Antibodies which allow targeting tumor cells have attracted increased interest within the last years. While a direct anti-tumor effect is unlikely and the same limitations as for CAR T cells regarding the tumor-specific expression of target antigen exist, antibodies may be coupled to a toxin or cytotoxic drug. Upon binding to a tumor cell, internalization of the immunoconjugate may result in a tumor-specific action of its payload. ABT-414 is an antibody which binds to amplified or mutant EGFR and is conjugated to monomethylauristatin. A phase I study using ABT-414 alone or in combination with temozolomide in patients with recurrent glioblastoma revealed unique corneal toxicity as a major side effect [66]. The drug is now being evaluated in 2 larger trials in patients with newly diagnosed or recurrent glioblastoma (NCT02573324, NCT02343406).

Conclusion and outlook

Despite various attempts within the last decades, immunotherapy has been largely inactive against various types of cancers including brain tumors. However, the field has made significant progress within the last years and several immunotherapeutic agents such as the checkpoint inhibitors have been approved for clinical use. There is increasing evidence that these drugs are also active against tumor manifestations in the brain. Whether the success story in the melanoma field can be translated into clinical benefit for patients suffering from glioblastoma and other primary brain tumors needs to be proven in ongoing trials. Beyond the checkpoint inhibitors, several “next-generation” vaccines are at late-stage clinical development. Even more advanced immunotherapeutic strategies comprise patient-tailored vaccines which are generated following a comprehensive analysis of the patient’s tumor tissue using large-scale screenings as well as the combination of vaccines with PD-1 inhibitors or other drugs that may overcome the immunosuppressive microenvironment.

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